FDA-NCI Roundtable: Symposium on Flow Cytometry Based Detection of Minimal Residual Disease in Multiple Myeloma

March 24, 2014
FDA White Oak Campus, Building 66 Room G258
10903 New Hampshire Avenue
Silver Spring, Maryland

AGENDA

This symposium will provide a forum for (1) the discussion of the current consensus standardization of the flow cytometric detection of minimal residual disease (MRD) in multiple myeloma (MM). And (2) what is the available data concerning the clinical significance of MRD in MM.

8:00 a.m.	Welcome and Symposium Objectives Summary of FDA-NCI Meeting at NIH, August 2013 Current *In vitro* Diagnostics Overview Devices	**Gerald Marti, M.D., Ph.D.** Medical Officer Immunology and Flow Cytometry Branch DIHD, OIR, CDRH, FDA
8:20	CDER Clinical Overview MM: Risk Benefit Determination and Current Therapies	**Nicole Gormley, M.D.** Medical Reviewer Division of Hematology OHOP, OND, CDER, FDA
8:30	Overview MM: Assessing Response in Clinical Trials and Imaging	**C. Ola Landgren, M.D., Ph.D.** Senior Investigator Center for Cancer Research, NCI
8:40	Limitations of Morphology: MRD Using H & E, IHC	**Constance M. Yuan, M.D., Ph.D.** Staff Clinician Flow Cyometry Unit Center for Cancer Research, NCI
9:00	*Clarifying Questions for the Speakers*	

9:10	Morning Panel: Review of Clinical Studies Demonstrating MRD as a Clinical Response Biomarker	**Nicole Gormley, M.D., (Chair)** Medical Reviewer Division of Hematology OHOP, OND, CDER, FDA
	UK clinical Flow MRD Experience	**Roger Owens, M.D.** St. James's University Hospital Leeds, UK
	Should MRD testing become a standard of care in multiple myeloma ?	**Bruno Paiva, M.D.** Flow Cytometry Core University
	Key Aspects of MRD Testing	**Brian Durie, M.D.** Cedars-Sinai Outpatient Center Los Angeles, CA
	Need for a sensitive method for MRD Assessment in MM: The lymphoSight Methodology	**Prof. Hervé Avet-Loiseau, M.D.** Unité de Génomique du Myélome CHU Rangueil, Toulouse
	The Utility of Morphology, Immunohistochemistry, Flow Cytomety and FISH Anaylsis in Assessement of Plasma Cell Neoplasm in the Bone Marrow	**Dr. Ahmed Dogan, M.D., Ph.D**. Chief, Hematopathology Service Memorial Sloan-Kettering Cancer Center New York, New York
10:25	*Break*	
10:40	Morning Panel Discussion	
12:00	*Lunch*	
1:00	Technical Requirements for MRD Analysis: Past, Present, and Future	**Andy Rawstron, Ph.D.** Consultant Clinical Scientist HMDS; Department of Haematology St. Jame's Institute of Oncology Bexley Wing, Beckett Street Leeds, LS9, 7 TF, UK

1:20	Afternoon Panel: Status of Consensus Standarization Guidelines For Flow Cytometry Assay	**Danielle Turley, Ph.D. (Chair)** Scientific Reviewer IMFB,DIHD,OIR,CDRH,FDA
	Staining and Acquisition in Flow Cytometric Myeloma MRD Testing	**Maryalice Stetler-Steveson, M.D., Ph.D.** Head, Flow Cytometry Unit, NCI
	Analysis and Reporting in Flow Cytometric Myeloma MRD Testing	**Maria Arroz, M.D.** Director of Flow Cytometry Laboratory, CHLO, Hospital S. Francisco Xavier, Lisbon, Portugal
	Quality Control in Flow Cytometric Myeloma MRD Testing	**Paul Wallace, Ph.D.** Professor of Oncology Director Department of Flow & Image Cytometry Roswell Park Cancer Institute
	Validation of Flow Cytometric Myeloma MRD Testing	**David Barnett** Consultant Clinical Scientist (Haematology) & Scientific Director UK NEQAS for Leucocyte Immunophenotyping Sheffield S10 2QD England
3:20	*Break*	
3:40	Afternoon Panel Discussion	
4:40	Wrap-up/Next Steps: Where do we go from here to make it a reality? Discussion: opinion paper	
5:00 p.m.	*Adjourn*	

Multiple Myeloma Clinical Overview and Risk Benefit Determination

Nicole Gormley, MD

Multiple Myeloma is a neoplastic proliferation of a single clone of plasma cells that produce a monoclonal immunoglobulin. It is estimated that there will be 24,050 new cases and 11,090 deaths from multiple myeloma in the United States in the year 2014 (Siegel *et al.*, 2014). Multiple myeloma is primarily a disease of the elderly, with a median age at diagnosis of 66.

The clinical features of multiple myeloma are a consequence of the proliferation and accumulation of clonal plasma cells or damage from excess light chains. Patients may present with signs and symptoms of anemia, bone pain or pathologic fractures, renal insufficiency, fatigue, hypercalcemia, or weight loss. The International Myeloma Working Group (IMWG) has developed standardized diagnostic criteria for multiple myeloma. To establish a diagnosis of symptomatic multiple myeloma, all 3 of the following criteria must be met: presence of M-protein in the serum or urine, presence of clonal plasma cell in the bone marrow or a plasmacytoma, and the presence of related organ or tissue impairment (Group, 2003).

Treatment options for multiple myeloma have significantly improved over recent decades with the introduction of alkylating agents, the use of high-dose therapy in combination with autologous stem cell rescue, and the introduction of new classes of agents such as immunomodulatory agents and proteasome inhibitors.

Despite these advances, patients with multiple myeloma often relapse or develop refractory disease, underscoring the need for new therapies. Additionally, with the advances seen in multiple myeloma and improvements in survival, the use of overall survival as a trial endpoint could result in prolonged drug development times. The U.S. FDA requires that for a new drug to be approved, the application must contain substantial evidence of efficacy with demonstration of acceptable safety in adequate and well controlled studies. The FDA examines the evidence in the context of the disease state, available therapy, study design, endpoints selected, and the strength of the evidence. Ultimately, the application must contain enough information to allow for the generation of a product label that defines an appropriate patient population, and provides adequate information for the safe and effective use of the drug. Various FDA programs have been designed to facilitate and expedite the review of new drugs. One such program, Accelerated Approval, is an approval pathway for products designed to treat a serious or life-threatening condition that have demonstrated an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit or an effect on a clinical endpoint that can be measured earlier than an effect on irreversible morbidity or mortality. The product must provide a meaningful benefit over available therapies and approval is usually contingent on the sponsor's agreement to conduct additional post-approval studies to describe and verify clinical benefit.

A biomarker is a type of surrogate endpoint and can be objectively measured as an indicator of a normal biologic process or response to intervention. When developing a biomarker to use as a regulatory endpoint, it is important to have a firm understanding of the underlying disease process, how the

biomarker fits in that process, and the effect of the intervention on both the disease process and the biomarker. The intervention may have a differential effect on the true clinical endpoint, and the biomarker endpoint, which may diminish the strength of the biomarker as a surrogate.

For minimal residual disease (MRD) to be used as a surrogate endpoint, there must be a clear understanding of the disease process, and the effect of the intervention on both the clinical benefit endpoint and the biomarker. It is important to understand if there is a difference in the effect on the biomarker with different interventions and if there are other factors involved which may diminish the correlation of the biomarker and the clinical benefit endpoint. Also, standardization of the definition and assessment methods for MRD is necessary for wider implementation, generalizability, and interpretation of the results generated.

References:

GROUP, I. M. W. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. **Br J Haematol,** v. 121, n. 5, p. 749-57, Jun 2003. ISSN 0007-1048. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12780789 >.

SIEGEL, R. et al. Cancer statistics, 2014. **CA Cancer J Clin,** v. 64, n. 1, p. 9-29, 2014 Jan-Feb 2014. ISSN 1542-4863. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24399786 >.

Multiple Myeloma: Assessing Response to Therapy in Clinical Trials

Ola Landgren, MD. PhD. Multiple Myeloma Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

ABSTRACT

In the multiple myeloma field; similar to other areas of clinical medicine, over time various response criteria have been used to assess clinical outcomes in relation to therapy. Almost a decade ago, the first version of the "International Uniform Response Criteria for Multiple Myeloma" was released (Durie et al, Leukemia, 2006). These criteria were based on "The European Group for Blood and Bone Marrow Transplant/International Bone Marrow Transplant Registry" criteria, which were expanded, clarified and updated to provide a new comprehensive evaluation system. For example, categories for stringent complete response and very good partial response were added. Also, the serum free light-chain assay was included to allow evaluation of patients with oligo-secretory disease. The International Uniform Response Criteria for Multiple Myeloma have been updated since and they are widely used in clinical trials.

Over the past decade, the proportion of multiple myeloma patients achieving a deeper response to therapy has gradually increased as newer and more effective therapies have become available. For example, older therapies such as melphalan/prednisone provided almost no patients with a complete response; combination chemotherapy followed by high-dose melphalan/autologous stem-cell transplant (ASCT) allowed up to 30-40% of patients to obtain a complete response; and newer drugs approved within the past few years suggest that up to 50-75% of patients may obtain a complete response even in the absence of high-dose melphalan/ASCT and many of these are in stringent complete response.

As expected, clinical studies show that multiple myeloma patients reaching a deeper response (vs poorer response) to therapy have a better progression-free survival and overall survival. Interestingly, recent studies focusing on patients achieving a complete response after having received combination chemotherapy followed by high-dose melphalan/ASCT show that about 30% have no detectable minimal residual disease (MRD) based on high-quality flow-cytometry assessment of bone marrow aspirates. Furthermore, these studies show that, within the group of patients who achieve a complete response, those that are MRD negative (vs MRD positive) have significantly better progression-free survival and overall survival. Based on small numbers, emerging data retrieved from patients treated with newer drugs approved within the past few years suggest that 75-100% of the patients who achieve a complete response become MRD negative as determined by high-quality flow-cytometry assessment of bone marrow aspirates.

Based on small numbers, molecular assays including ASO-PCR and deep sequencing of the VDJ sequence have been reported to correlate with high-quality flow-cytometry and also be able to predict progression-free and overall survival in myeloma. In addition, molecular imaging has been reported to play a role in the determination of MRD status after delivery of anti-myeloma therapy. Recently, a clinical trial reported residual elevated SUV values detected by PET/CT (ie, pre-therapy vs post-therapy) to be predictive of clinical outcomes. Future studies are needed to validate and expand currently available data regarding molecular MRD assays and molecular imaging.

Independent of the preferred methodological platform(s) for evaluation of response to treatment, based on the success of newer drugs it seems reasonable to argue that with more active agents becoming available, there is emerging need to assess not just if complete response has occurred, but the exact magnitude of response and if there is any evidence of MRD. Given that virtually every study focusing on MRD testing in myeloma has reported MRD negativity (vs MRD positivity) to be associated with better progression-free survival, and some studies have found MRD negativity to be associated with better overall survival, it seems logical to propose that MRD testing needs to be integrated in future uniform response criteria for multiple myeloma, and, consequently, should be considered for regulatory purposes including drug approval in the field of multiple myeloma. In order to facilitate this process, there is urgent need for consensus criteria for MRD negativity in myeloma.

Limitations of Conventional Diagnosis of Myeloma and Myeloma Residual Disease by morphologic methods.

Constance M. Yuan, MD PhD. NCI, NIH, Bethesda, MD.

Conventional diagnosis of myeloma and detection of myeloma residual disease rely significantly on plasma cell enumeration, often by morphologic means. The aspirate smear and core biopsy each provide information about plasma cells that are slightly different, but complementary. If cytologic atypia is present, the aspirate smear readily identifies the presence of abnormal plasma cells. The core biopsy provides less cytologic detail than the aspirate smear, but can assess the pattern (interstitial, small clusters, nodular, diffuse) and degree of marrow infiltration and determine clonality, when immunohistochemistry and in-situ hybridization methods are used in conjunction with H&E. Nevertheless, these methods are not without their limitations.

Diagnosis of plasma cell myeloma includes plasma cell enumeration (among other criteria, according to the WHO Classification), traditionally obtained from manual counts of the aspirate smear, despite the observation that plasma cells are often not uniformly distributed in these preparations. Furthermore, morphologic methods may suffice for diagnostic purposes when plasma cells are abundant, but may be less useful after treatment when plasma cells are few. Additionally, inter-observer variability remains an obstacle, although this is improved with the use of immunohistochemistry on the core biopsy. Finally morphologic methods cannot consistently distinguish abnormal from normal plasma cells. Normal plasma cells may be observed after treatment and their presence plays a role in prognosis.

The utility and limitations of morphologic assessment in diagnosis of myeloma and myeloma residual disease need to be understood and recognized. Information from other laboratory technologies/methods may be needed to supplement the information provided by morphology.

Minimal residual disease (MRD) in myeloma: the UK experience.

Roger G Owen, Ruth M de Tute & Andy C Rawstron.
HMDS Laboratory, St James's Institute of Oncology, Leeds, UK.

The assessment of MRD using multiparameter flow cytometry (MFC, sensitivity 10-4) has been performed in sequential Medical Research Council (MRC) trials since the late 1990s. The impact of MRD has been evaluated in Myeloma VII, Myeloma IX, Myeloma X and Myeloma XI trials and >1000 patients have thus far been evaluated. The following conclusions (at least in the context of the transplant eligible population) can be made from our studies

- MFC is informative in ~97% of patients. This is likely to be the theoretical maximum as the majority of non-informative patients have non-representative marrow samples rather than "normal" plasma cell immunophenotypes.

- MRD at day 100 post ASCT is predictive of outcome – PFS and OS. This effect is seen in both upfront and salvage ASCT settings.

- MRD predicts outcome in CR patients.

- MRD predicts outcome in patients with both high and standard risk cytogenetic profiles.

- The prognostic effect of the post ASCT assessment is independent of the induction therapy received. This is very similar to data produced in CLL, which suggests that it is the quality of response, however achieved, is the determinant of outcome and not the specific therapy received.

- The MRC outcome data is broadly comparable to that published by colleagues from the Spanish group.

- MRD is likely to become more relevant in transplant ineligible patients as therapies continue to improve.

- Sequential MRD assessments allow for a more definitive assessment of the effect of induction and consolidation / maintenance strategies when changes in categorical M protein response can be difficult to demonstrate. In this context we have been able to demonstrate further plasma cell depletion following ASCT with maintenance thalidomide.

- Current outcome data is based upon a sensitivity of detection of 10-4. As therapies improve a greater level of sensitivity will be required. Nevertheless the presence of residual disease at the 10-4 level, regardless of therapy received, defines a patient population with an inferior outcome.

- Our ongoing clinical trial activity will focus on the following areas

- further assessment of outcome prediction post ASCT in the Myeloma XI/XI+ trial which will include a comparison of MFC with novel sequence based methodologies
- assessment of the effect of lenalidomide maintenance on MRD in both transplant eligible and ineligible groups
- assessment of carfilzomib consolidation in the relapse setting
- randomized trial of adjuvant systemic therapy in patients with high risk plasmacytoma of bone

Bruno Paiva, MD

Should MRD testing become a standard of care in multiple myeloma?

The use of immunophenotypic and molecular techniques to evaluate response to therapy has become standard practice in many hematological malignancies. By contrast, up until now response to treatment in multiple myeloma (MM) has only been evaluated by conventional clinical, morphological and serological parameters. In MM continuous efforts are being made to improve the efficacy of therapy, which translates into a decreased number of residual tumour cells after therapy. Thus, unprecedented rates of complete remission (CR) are now being achieved after up-front treatment and overall survival has significantly improved; however, only a minor fraction of patients actually achieves long-term disease control (>10 years disease-free survival) which underlies the presence of minimal residual disease (MRD) undetectable by conventional techniques. Accordingly, MRD detection by multiparameter flow cytometry (MFC) immunophenotyping has consistently proved to be capable of identifying two subgroups of patients in CR with significantly different outcome: those achieving Flow-CR and those with persistent MRD. Importantly, these findings are observed throughout the whole landscape of MM, ranging from the younger to the elderly patients, after first-line or salvage therapy, and applying to both standard- and high-risk cytogenetically-defined patients. The experience accumulated in different clinical trials has shown that the detection of MRD specifically among patients in CR is consistently associated with a 2-fold reduction in median time-to-progression, and particularly among transplant-eligible patients, also significantly reduced overall survival. Altogether, the published results validate the clinical relevance of the limit of detection of conventional MFC (10^{-4}); importantly though, the availability of polychromatic and faster flow cytometers as well as new software and analytical approaches should build up on this, reach a higher limit of detection and quantitation (10^{-5}), and provide the tools for comparable and reproducible results among laboratories and clinical trials.

MRD and Strategies to Achieve Cure: The IMF Black Swan Research Initiative®

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The Black Swan Research Initiative (BSRI®) is a global collaborative approach which focuses on the use of the best MRD testing to track myeloma at the lowest levels as a basis for treatment decisions to achieve cure. Although the quantitative impact remains to be established, MRD testing clearly adds to outcomes assessment. To integrate MRD testing into routine clinical practice, there are several key requirements.

1. A reproducible, standardized test
2. Sensitivity with results quantitative at 10^{-5} level or better
3. A method which is widely available and cheap
4. Rapid turnaround; preferably local/center testing
5. Ability to detect all subclones
6. Results which can direct treatment decisions
7. Integration into IMWG guidelines and FDA drug/treatment review/approvals

The new multiparameter flow method developed by the Spanish team (Universities of Salamanca and Navarra) satisfies these requirements. By comparison, currently available molecular methods, including the Sequenta DNA method, are less optimal for these needs. The main issues are that the DNA methods do not detect all potential clones: the "dominant" clone is identified for subsequent monitoring. Sensitivity is good, but testing is not as accessible or cheap as flow. Samples are sent off with a 10-14-day turnaround, which negatively impacts potential decision making. This also means that it is not realistic to propose any current DNA testing as a global standard within IMWG guidelines and/or for FDA review processes.

The new flow method by contrast is attractive for multiple reasons. A central component is the software/analytic programming which provides a standardized objective computer readout. This software can also be adapted to several models of multiparameter flow cytometry machines. It is also key that flow assessment can provide several "readouts" of different types of information such as: MRD-Zero (no MRD detected); quantitation of MRD at 10^{-5} level; identification of an MGUS signature; identification of an MDS signature; identification of subclones which can be further characterized; and analyses of microenvironmental cell patterns.

It thus becomes obvious that effective MRD testing can contribute to better QOL and outcomes for patients, improved assessment of response in clinical trials and potentially a more quantitative/standardized regulatory review process which can speed up drug development. With this in mind, broad endorsement of agreed methods and strategies is to be sought as a top priority.

Need for a sensitive method for MRD assessment in MM: the LymphoSight approach

Hervé AVET-LOISEAU, MD, PhD, Unit for Genomics in Myeloma, Institut Universitaire du Cancer, Toulouse, France.

In multiple myeloma (MM), most of clinical trials are taking for primary endpoint the PFS. However, this endpoint is not anymore valid in MM. The main reason is that patients are currently living for a much longer time after the first relapse than in first response. For instance, in young patients, the median first PFS is around 3-4 years, whereas the median OS is exceeding 10 years. This fact has been also identified by the health authorities (FDA, EMA), who do not accept anymore studies based on PFS for drug approval. Thus we need surrogates markers of OS to design trials interpretable in a reasonable period of time. Minimal Residual Disease (MRD) evaluation could represent an interesting approach to achieve this goal.

In order to address this issue, we conducted MRD studies in the recent joined IFM/DFCI trial. Briefly, this trial randomized 700 patients in IFM and 650 patients in US, half receiving an 8-RVD treatment before Lenalidomide maintenance, and half receiving 3 courses of RVD, high-dose Melphalan with ASCT, 2 courses of consolidation RVD, and then maintenance. All patients achieving at least VGPR were evaluated for MRD. A fraction of the MRD samples were analyzed by flow cytometry, and the rest was frozen for molecular assessment of MRD using the LymphoSight technology developed by Sequenta. For flow cytometry, we used a 7-color single tube (CD45, CD19, CD38, CD138, CD56/CD28, Kappa, Lambda), with a sensitivity of 10^{-4} for all the patients. The CR/nCR rate before maintenance was 64%. If restricted to patients in CR, MRD was positive in only 12% of them. We think that this information is definitely not sufficient to identify the patients who will have a long survival. We definitely need more sensitive tools to reach this aim. More sensitive flow cytometry protocols, or molecular analyses may be the optimal approach. All our patients are currently analyzed by Sequenta, Inc.

The main advantage of PCR-based methods is their sensitivity, in the 10^{-6} range. This technique has also several disadvantages, if compared to flow cytometry: requirement for clone identification (effective in 70% of the patients), design of specific primers, time (and money). The development of NGS-based approaches seems to resolve most of these pitfalls. A recent study by the Spanish group using the LymphoSight platform analyzed 133 patients. A clonal rearrangement was found in 91% of them. In these patients at least in VGPR at the time of analysis, 73% remained MRD positive by NGS (58% if restricted to patients in true CR). When compared to flow data, 61% of the patients were positive by the two methods, 22% were negative, and 17% were discordant, mainly positive by flow cytometry and negative by NGS. The TTP was shorter for those 17% of patients as compared to patients negative by NGS.

In conclusion, NGS approaches are promising in MM to identify patients who are really good candidates for long-term survival.

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The Utility of Morphology, Immunohistochemistry, Flow Cytometry and FISH Analysis in Assessment of Plasma Cell Neoplasm in the Bone Marrow

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Background: Bone marrow (BM) evaluation of plasma cell (PC) disorders requires enumeration of PC and demonstration of clonality. Enumeration of the PCs can be performed in the BM aspirate, by immunohistochemistry (IHC) for CD138 in the BM biopsy, or by flow cytometry (FC). Clonality can be demonstrated by establishing light chain (LC) restriction of the PC in BM biopsy by IHC, LC restriction and/or abnormal phenotype by FC or cytogenetic abnormalities. A direct comparison of the utility of multiple testing modalities in accomplishing these goals has not been previously reported from a US-based study, we sought to compare these modalities for the evaluation of PC neoplasms.
Design: 100 consecutive BM samples submitted for evaluation of PC neoplasms were studied through H&E stained biopsy cores, IHC for CD138 and LC, Wright-Giemsa stained aspirates and highly sensitive FC. Multiple myeloma (MM)-specific fluorescence *in situ* hybridization (FISH) was performed on PC-enriched BM cells. All 100 samples had morphology with corresponding FC analysis, 95 had an evaluable aspirate smear and 86 had myeloma FISH. Morphologic evaluation was judged positive when greater than 5% PC were present in the BM aspirate or biopsy and clonality was established by IHC. FC positivity required demonstration of at least 50 LC- restricted PCs showing abnormal immunophenotype. FISH was judged positive when MM specific chromosomal abnormalities were detected.
Results: 93 patients had an established diagnosis of PC neoplasm, while 7 cases were new submissions. Of the 100 samples, 81 demonstrated clonal PC proliferation. CD138 stain yielded the highest estimates of the plasma cell proportion compared to aspirate count or flow cytometry ($p<0.01$) with mean values of 30% (range: 3-100), 19 (1-78) & 2.1 (0.01-33.2) respectively. The biopsy proportion estimate correlated well with the aspirate count ($r^2=0.6$), but poorly with FC evaluation ($r^2= 0.1$). FC demonstrated the highest sensitivity in assessment of clonality 96% (78/81), while morphology+IHC was positive in 84% (68/81), followed by MM FISH/Cytogenetics 79% (59/75). Two cases were positive on morphology while negative by FC due to clotted FC samples.
Conclusions: Our results show that IHC for CD138 is the most sensitive method for assessment of PC numbers in the BM specimens involved by a PC neoplasm. In contrast FC immunophenotyping is the most sensitive method to establish the clonal nature of the BM PC.

Technical requirement for MRD analysis: past, present and future

Andy C. Rawstron, HMDS, Leeds Teaching Hospitals NHS Trust, UK.

Myeloma is currently incurable but overall survival is typically greater than five years for treatment-naïve trial-eligible patients. New and effective treatments are being developed at an unprecedented rate but randomized phase 3 trials take several years to assess survival end-points. Response assessment is currently based on morphological assessment of a bone marrow biopsy, which has very limited sensitivity, coupled with changes in serum monoclonal immunoglobulin (paraprotein) levels. Due to the high degree of inter-patient variability in the amount and half-life of secreted paraprotein, current response assessment can only provide an indirect measure of a relative change in tumour burden.

The use of molecular and/or flow cytometry assays to quantify the burden of neoplastic plasma cells in the bone marrow provides a more sensitive and direct measure of response. Although molecular approaches offer higher sensitivity, large trials demonstrating a survival benefit for MRD-negative patients have used flow cytometry. The advantages of flow include applicability to the vast majority of patients using a standard assay, direct quantification with the same limit of detection and quantification in every case, and the ability to assess sample quality within the assay so that false-negative results can be automatically excluded. Studies with effective prediction of outcome have used a strategy consistent with the 2008 European Myeloma Network consensus document, with a set of markers to identify plasma cells (CD138/CD38/CD45) and a further set to differentiate neoplastic from normal (CD19/CD56/CD117/CD27). Clonality (Kappa/Lambda) assessment has limited value in MRD analysis and may give confounding results. There are three prospective studies showing that the level of residual disease, using a 0.01% limit of detection, is an independent predictor of progression-free and overall survival. Data from the UK MRD myeloma IX trial demonstrates approximately one year improvement in overall survival for each log reduction in tumour burden.

The development of high throughput sequencing (HTS) provides a promising alternative to flow cytometry. The approach can detect disease at the 0.0001% level, although in practice the limit of quantification is likely to be 0.001%. Unlike previous molecular approaches, HTS strategies can use the same set of primers for all patients and such assays are now commercially available. HTS requires prospective validation and there are issues with quantification to be resolved, including the calibration and correction approaches used to determine total leucocytes and B-lineage cell numbers. Other conceptual issues such as clonal heterogeneity, clonal evolution and selection under treatment, or concomitant clonal B-lineage disorders remain to be addressed. While further validation of HTS is ongoing, ICCS and ESCCA have recognized the need for a harmonized flow cytometry approach which provides backwards compatibility with established assays but also offers sufficiently high sensitivity to remain relevant for the next decade as treatment for myeloma evolves.

There are benefits and disadvantages to all of the current MRD detection strategies but there is clear evidence from previous studies that the level of residual disease, based on a 0.01% threshold, is a strong independent predictor of survival and could be an appropriate clinical trial end-point. Current technologies make MRD detection more rapid and straightforward and 0.001% is now a reasonable limit of quantification by flow cytometry and HTS, with HTS potentially identifying patients achieving less than 1 myeloma cell per million normal cells. In order to achieve accurate quantification with maximum sensitivity and no false negative results it is optimal to combine both strategies. As therapies improve there will be an increasing need for validated, sensitive and directly quantitative analysis of bone marrow tumour burden to identify differential efficacy of specific agents in multi-component treatment strategies.

Guidelines for Staining and Acquisition in Flow Cytometric Myeloma MRD Testing:

Maryalice Stetler-Stevenson, M.D., Ph.D., NCI, NIH, Bethesda, MD

The quality of flow cytometric myeloma MRD testing is dependent upon the specimen quality and on the staining and acquisition process. An international group of experts developed a set of guidelines for staining and acquisition in myeloma MRD testing based upon extensive experience using protocols validated in clinical trials. At present bone marrow (BM) specimens are the standard for flow cytometric MRD assessment. Samples should be stained within 24 hours (48 hour cut off) and viability should be greater than 85%. Since clonal PCs are present in very low numbers in post-treatment BM, red cell lysis and concentration of cells should be performed first to deliver 3 to 5 million cells per 100 to 200uL volume for the staining process. The consensus on best practice for detection of MRD in myeloma requires study of the following antigens: CD38, CD138, CD45, CD19, CD56, CD27, CD81, and CD117. Intracellular light chain evaluation does not provide additional information in greater than 97% of patients. In addition to abnormal plasma cells, the panel must be able to assess the quality of the aspirate. Routine usage of an identical panel in all cases is highly recommended. Each panel must be tested extensively to determine optimal fluorochome usage and it is recommended that laboratories initiating myeloma MRD testing adopt a validated panel. Acquisition is successful when MRD is detected or the acceptable minimum total cell collection is achieved AND the specimen meets criteria for quality. Previous studies demonstrating the clinical relevance of an immunophenotypic complete response were based upon acquisition of 500,000 events. Therefore an absolute minimum of 500,000 events is required. Ongoing studies indicate that higher numbers of acquisition are indicated for best practice. It is therefore consensus that two million events is the acceptable minimum total cell collection in the absence of MRD and that 3 to 5 million may be optimal. If fewer than 2 million events are acquired and MRD is not detected the LOD should be stated and a qualifying statement as to the decreased level of sensitivity placed in the report. Implementation of these guidelines across institutes should assure the quality and comparability of myeloma MRD testing in clinical trials.

Maria Arroz

Analysis and reporting of MRD in MM

Major heterogeneity between laboratories in flow cytometric Minimal Residual Disease testing in Multiple Myeloma is a reality. Cytometry societies such as the International Clinical Cytometry Society (ICCS) and the European Society for Clinical Cell Analysis (ESCCA) felt a strong need to establish minimum requirements and recommendations to perform such complex testing. An international group of experts in the field developed guidelines for analysis and reporting of myeloma MRD across laboratories despite different instruments, variable myeloma MRD panel designs (≥ 6-color) and softwares used. There is strong consensus to employ a minimum of four gating parameters (CD38, CD138, CD45 and light scatter) within the same tube for the identification of the total plasma cell compartment, with subsequent analysis of potentially aberrant surface markers, which is almost always more informative than cytoplasmic light chain analysis. Reporting an antigen expression pattern on neoplastic plasma cells as being reduced, normal or increased, compared to normal reference plasma cell immunophenotype (obtained using the same instrument and parameters), including the percentage of positive cells for each marker, is important to establish the immunophenotypic signature that will aid in follow-up MRD detection.

The consensus is that current and future MRD analyses should target a lower limit of detection of 0.001%, which requires at least 3 $x10^6$ bone marrow cells to be acquired, and ideally a limit of quantification of 0.001%, which requires at least 5 x 10^6 bone marrow cells to be acquired.

The proportion of the total plasma cell pool defined by flow cytometry as neoplastic, or conversely the percent normal residual, or re-emerging, plasma cells prior to or following therapy is of high prognostic relevance and should always be reported, because the balance between phenotypically aberrant and normal plasma cells is not likely to be affected by hemodilution.

Quality Control in Flow Cytometric Myeloma MRD Testing

Paul K Wallace, PhD, Roswell Park Cancer Institute, Buffalo, NY 14263

Quality control (QC) is a continuous process that monitors the stability of the entire testing process over time and is critical for accurate, high sensitivity myeloma MRD testing. QC includes all components of the assay: reagents, instrumentation, operator skills and environmental factors. The purpose of a good QC process is to monitor and verify that the accuracy and precision of the MRD assay does not deviate from the performance criteria established during the validation or verification process.

Instrument QC consists of the initial instrument setup, a maintenance program and performance monitoring over time. Instrument performance must be verified daily, at the start of each shift, and is routinely done with stable latex beads which should fall into established targets channels for each parameter. This will assure that identical fluorescent and light scatter signals are obtained over time. This is especially critical for myeloma MRD testing as subtle differences in fluorescent and/or light scatter are used to distinguish normal from abnormal populations. Compensation settings, used to subtract the spectral overlap between the different fluorochromes, are established at assay validation and should be verified daily using either stained cells or antibody capture beads. They should be optimized after instrument maintenance or anytime voltage settings are adjusted significantly during the setup process.

Antibody reagents in the US are designated as either ASR (Analyte Specific Reagent) or IVD (*In* Vitro Diagnostic). IVD reagents are used as the manufacturer recommends whereas new lots of ASR reagents should be titered and parallel tested with the current lot. All of the reagents for myeloma MRD testing are ASR at this time and must be validated by the laboratory. The use of premixed antibody cocktails in the myeloma MRD assay can simplify and standardize the assay set up and minimize potential errors in pipetting. If a laboratory utilizes cocktails for the assay, validating that the results are equivalent for the cocktail over time is required.

Samples for flow cytometric analysis generally include blood and bone marrow aspirates. Accurate MRD testing requires that the sample integrity be maintained and a well designed antibody panel will assess the quality of the sample. This can be done through demonstration and phenotyping of normal plasma cells and assessment of neutrophil maturation. It is recommended that each analysis include a reported viability test and that samples with viabilities of less than 85% should be rejected.

Operator training provides the technologist with knowledge and skills required to perform all aspects of myeloma MRD testing. This may include lectures or tutorials, SOP's, practice of the task with a skilled observer, testing blinded samples and analysis of listmode files. While there are numerous methods that can be used for training in the end competency at performing all aspects of myeloma MRD testing must be verified and documented.

After validating the myeloma MRD test, a QC program that takes into consideration all of these variables is essential for accurate and reproducible results especially for the lower limits of detection required for MRD testing. It is essential to have optimized and standardized procedures, rigorous quality control and assurance programs encompassing preanalytic, analytic, and postanalytic processes.

David Barnett Abstract:

Flow cytometry and other technologies of cell-based fluorescence assays are as a matter of good laboratory practice required to validate all assays, which when in clinical practice may pass through regulatory review processes using criteria often defined with a soluble analyte in plasma or serum samples in mind. Recently the U.S. Food and Drug Administration (FDA) has entered into a public dialogue in the U.S. regarding their regulatory interest in laboratory developed tests (LDTs) or so-called “home brew” assays performed in clinical laboratories. The absence of well-defined guidelines for validation of cell-based assays using fluorescence detection has thus become a subject of concern for the International Council for Standardization of Haematology (ICSH) and International Clinical Cytometry Society (ICCS). Accordingly, a group of over 40 international experts in the areas of test development, test validation, and clinical practice of a variety of assay types using flow cytometry and/or morphologic image analysis were invited to develop a set of practical guidelines useful to in vitro diagnostic (IVD) innovators, clinical laboratories, regulatory scientists, and laboratory inspectors. This talk will summarise these findings showing the requirements that the MRD myeloma group would expect all laboratories to use.

FDA-NCI Round Table 'Mini-Symposium'

Monday March 24, 2014
Building 66 Room G258
FDA White Oak

Meeting Roster

I. Foreign Visitors

1. Maria Arroz
2. Herve Avet-Loiseau
3. David Barnett
4. Andy Rawstron
5. Bruno Paiva
6. Roger Owens
7. Ruth De Tute

II. Non-foreign US Visitors

1. Ola Landgren
2. Maryalice Stetler-Stevenson
3. Paul Wallace
4. Ahmet Dogan MD PhD
5. Brian Durie
6. Connie Yuan
7. Irina Maric
8. Raul Braylan
9. Kathy Calvo
10. Roschewski, Mark J
11. Elisabet Manasanch
12. Dalia Salem
13. Heba Degheidy
14. Kazandjian, Dickran, FDA NCI
15. Neha Korde

III. CDER FDA

1. Gregory Reaman
2. Angelo de Claro
3. Anne Farrell
4. Nicole Gormley
5. Christine Lincoln
6. Edvard Kaminskas
7. Albert Deisseroth
8. Thomas Herndon

IV. CDRH FDA

1. Parkash Jha
2. Max Robinowitz
3. Robert Becker
4. Liz Mansfield
5. Maria Chan
6. Liz Stafford
7. Danielle Turley
8. Kevin Maher
9. G Marti
10. Donna Roscoe
11. Jenn Dickey

V. Lucy Bauer,